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An Affymetrix Microarray Design for Microbial Genotyping

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Technical Memorandum

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Abstract

There is an ongoing requirement for development of high-density or multiplex assays for detection or identification of microbes. There is also a need to develop assays or toolsets that can detect or identify microbial threats without prior knowledge of the target microbe(s) in a given sample. Indeed, some samples that contain no culturable material (e.g. viable but non-culturable cells) will nonetheless contain detectable DNA fragments which might be of value with respect to forensics or attribution of source. For many pathogenic microbes, various specific tests already exist, but there are few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms. The high-density DNA microarray has the potential to address many of these requirements and thus complements existing identification tools. The microarray platform has for example, the ability to detect microbial DNA that is not a perfect match to known genomic DNA sequences, thus making it possible to detect microbial variants that might otherwise be missed. In this report, the design and preliminary testing of a high-density DNA microarray for the purpose of microbial identification and detection is described.

Résumé

Les méthodes à haute densité et les techniques multiplex pour la détection et l’identification des microorganismes sont des outils toujours en demande. Il faut aussi des méthodes pour détecter et identifier les dangers microbiens savoir quels microorganismes peuvent être présents dans les échantillons. En fait, certains échantillons ne contenant que des espèces non cultivables (c.-à-d. qui sont viables mais qui ne peuvent être mises en culture) peuvent contenir des fragments d'ADN détectables qui pourraient être utiles à des fins criminalistiques ou pour la détermination de l’origine du matériel. Diverses méthodes spécifiques existent déjà pour de nombreux microorganismes pathogènes, mais il y a peu de méthodes générales avec lesquelles une même technique adaptable peut être appliquée à de multiples espèces ou à des microorganismes qui n'ont pas été préalablement caractérisés. La puce à ADN à haute densité pourrait satisfaire à un bon nombre de ces critères et être un complément aux outils d’identification dont nous disposons actuellement. Elle peut servir, par exemple, à détecter l’ADN microbien qui ne correspond pas entièrement aux séquences d’ADN génomique connues, ce qui permet de détecter des variants microbiens qui, autrement, auraient pu passer inaperçus. Dans le rapport présenté ici, nous décrivons la conception et les essais préliminaires d’une puce à ADN à haute densité mise au point pour la détection et l’identification des microorganismes.

Executive summary

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Background: DNA and RNA (nucleic acids) are the genetic material of bacterial and viral species. The composition (e.g. DNA sequence) of the genetic material can be used to determine unambiguously, the identity of the species down to the level of individual unique strains. There are in existence assays useful for the detection and identification of single target nucleic acid sequences in microbial samples. In surveillance or diagnosis, it may be necessary to screen for many biological agents without knowing which in particular is of interest. While it is possible to run multiple single assays in parallel, the ability to execute multiple assays simultaneously within a single assay run is limited. Thus testing for multiple species of micro-organism currently requires multiple assay runs.

There is also a need to develop toolsets that can assay microbial targets without extensive microbiological culture or analysis of the specific sample. For example, some samples that can't be cultured at all will nonetheless contain detectable DNA fragments which might be of value with respect to forensics or attribution of source. There are currently few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms.

The high-density DNA microarray has the potential to complement existing identification tools, especially for multiple species or strains, or samples which can't be cultured using conventional microbiology. Microarrays are a technology which permits the detection of many nucleic acid sequences in a single run, with identification of each detected sequence. The basic microarray is comprised of many individual DNA sequence targets on a tiny microscope slide. The Affymetrix platform represents the current state of the art in microarray density (more than 221,000 individual targets) and throughput. An advantage of the microarray platform is the ability to detect microbial DNA sequences that are not a perfect match to the DNA sequences on the microarray chip.

Results: In this report, the design and preliminary testing of a high-density Affymetrix DNA microarray for the purpose of microbial identification and detection is described. The microarray can discriminate between multiple species of interest using qualitative analysis.

Significance: The DNA microarray is a single adaptable high-density platform useful for detection, identification and discrimination of multiple threat agents simultaneously. It is a complementary diagnostic technology to existing low-density microbiological and assay systems.

Future plans: Detailed validation of the current microarray and comparison to other microarray systems is planned. Additional testing is required to fully assess the real-world value of the DNA microarray as a tool for diagnosis, detection, and identification of microbial samples. Mixed microbial DNA samples and samples with human DNA (a frequent element in diagnostic samples) will be evaluated.

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An Affymetrix Microarray Design for Microbial Genotyping

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Contexte : L'ADN et l'ARN (acides nucléiques) constituent le matériel génétique des espèces bactériennes et virales. La composition (c.-à-d. la séquence des acides nucléiques) du matériel génétique peut servir à déterminer sans aucune ambiguïté l'identité d'une espèce jusqu'au niveau des souches individuelles uniques. Diverses méthodes servent à la détection et à l'identification de séquences d'acides nucléiques cibles dans les échantillons microbiens. Pour des besoins de surveillance ou de diagnostic, il peut être nécessaire de rechercher de nombreux agents biologiques sans savoir lesquels cibler plus particulièrement. Il est possible de faire plusieurs analyses parallèlement, mais peu de méthodes permettent de rechercher simultanément plusieurs microorganismes par une seule analyse. Ainsi, actuellement, lorsqu'il faut rechercher plusieurs microorganismes dans un échantillon, il faut le soumettre à plusieurs analyses.

Il faudrait aussi mettre au point des méthodes qui permettent de cibler des microorganismes sans qu'il soit nécessaire de faire des cultures ou des analyses avancées. Par exemple, certains échantillons ne se prêtant pas à la culture peuvent contenir des fragments d'ADN détectables qui pourraient être utiles à des fins criminalistiques ou pour la détermination de leur origine. Actuellement, il existe peu de méthodes générales avec lesquelles une technique adaptable peut être appliquée à de multiples espèces ou à des microorganismes qui n'ont pas été préalablement caractérisés.

La puce à ADN à haute densité pourrait être un complément utile des outils d'identification actuels, surtout pour les espèces ou souches multiples ou encore pour les échantillons qui ne se prêtent pas aux méthodes culturales de la microbiologie classique. La puce à ADN est une technologie qui permet la détection et l'identification de nombreuses séquences d'acide nucléique en une seule analyse. Essentiellement, une puce à ADN est une petite lamelle de microscope sur laquelle ont été déposées un grand nombre de séquences individuelles d'ADN cible. La plate-forme Affymetrix est actuellement le dernier cri de la technologie des puces à ADN à haute densité (plus de 221 000 cibles individuelles) et à débit élevé. L'un des avantages de cette plate-forme vient de ce qu'elle permet de détecter des séquences d'ADN microbien qui ne correspondent pas entièrement aux séquences utilisées sur la puce.

Résultats : Dans le rapport présenté ici, nous décrivons la conception et les essais préliminaires d'une puce à ADN à haute densité Affymetrix mise au point pour la détection et l'identification des microorganismes. Cette puce permet de différencier un grand nombre d'espèces d'intérêt par une analyse qualitative.

Importance : La puce à ADN est une plate-forme adaptable à haute densité qui peut servir pour la détection, l'identification et la différenciation simultanées de multiples agents dangereux. Cette technologie de diagnostic est un complément des systèmes de détection et d'analyse microbiologiques à faible densité.

À venir : Validation détaillée de la puce à ADN actuelle et comparaison avec d'autres systèmes à puce. D'autres essais seront nécessaires pour l'appréciation exacte de la valeur opérationnelle de la puce à ADN comme outil de diagnostic, de détection et d'identification des échantillons microbiens. Des échantillons d'ADN microbien mixtes et des échantillons contenant de l'ADN humain (dont la présence est fréquente dans les échantillons de diagnostic) seront évalués.

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1 Introduction

In the area of microbial genotyping there are multiple platforms that can identify one or a few microbial targets in a single assay iteration. For most pathogenic microbes, various specific methods exist, but there are few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms. There is a continuing need for the capability to detect or identify many possible microbial agents without having prior knowledge of the offending agent in a given sample [1,2]. Indeed, some samples that contain no live or culturable cells could contain detectable DNA fragments which may prove to be useful for clinical, forensic or attribution purposes [3]. One platform with potential to aid identification of multiple species or strains without culturing or specific prior sequence knowledge is the high-density microarray.

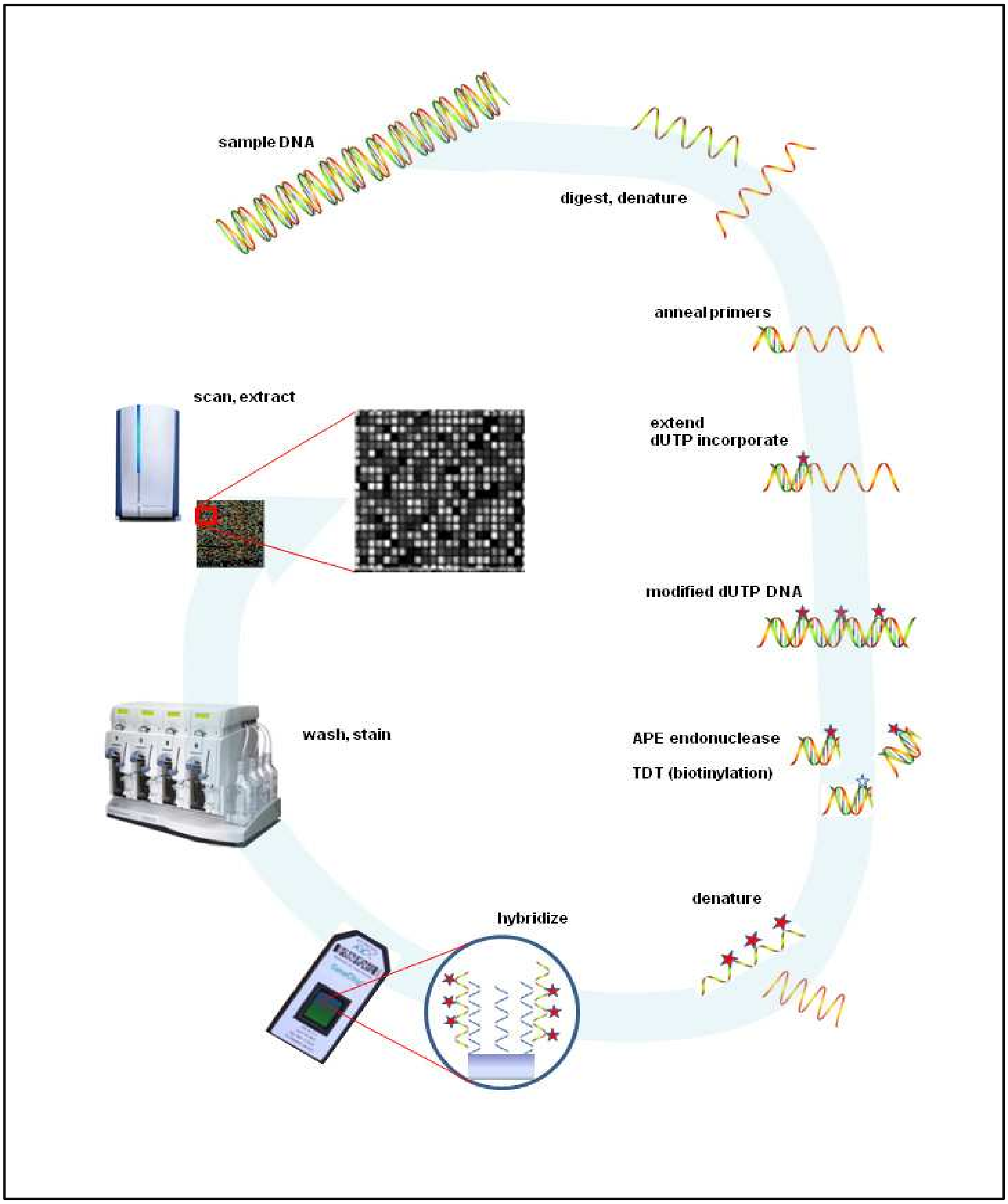
Each microarray can carry from a few hundred to a few hundred thousand individual target DNA sequences. Choice of specific array platform is driven by a combination of cost, density, and usability. For maximal utility, the ideal microarray should have as many features as possible, each feature representing one unique DNA sequence fragment. In the current work, the Affymetrix platform was exploited towards the development of a broad spectrum multi-species, multi-strain microarray, on a single microarray chip containing over 200,000 individual features. The Affymetrix system is closed source, meaning that the applied technologies for array fabrication, labeling, and data extraction are integrated into a pre-packaged system purchased from Affymetrix. Basic methods are established by the vendor, such that standardization of techniques takes much less time than with open source platforms. There are additional hardware costs for choosing Affymetrix relative to open source platforms, which are largely balanced by the reduced hands-on time for the pre-hybridization and post-hybridization manipulations, as well as for the extraction of data from the scanned microarray image. Figure 1 is a summary of the Affymetrix microarray processing system used at DRDC Suffield in support of this work. The protocol is itemized in detail in the Annexes.

In order to assess the utility of DNA microarrays for identification of bacterial pathogens to the species and strain level, a multipathogen chip was designed for the Affymetrix platform. Organisms included on the chip were derived from the National Institute of Allergy and Infectious Diseases Category A and B list of priority pathogens [4]. Also selected were *Haemophilus influenzae*, *Acinetobacter baumannii*, *Chaetomium* species, *Rickettsia* species, plasmids pBC16 and pLS1. Sequences representing bacterial toxins and antimicrobial resistance (*e.g.* antibiotic markers) were also sampled. Targets for viral pathogens were not included in this chip. The sequences thus chosen constituted approximately 16,000 individual sequence targets, which, allowing for sequence variants and internal controls, included over 81,000 unique probes. The remaining capacity of the chip surface was used to deploy some 140,000 probes from the Affymetrix "antigenomic library" to serve as non-targeted probes, essentially a random target library.

The number of microbial genomic targets thus did not equal the number of individual probes on the array. This is due to the redundancy built into the Affymetrix microarray technology, wherein variants of specific probe sequence differing by one or a few bases from the specific probe, are used to assess non-specific or variant binding to probe sites. In general, each specific target is represented by 3–20 individual probe sequences, varying by length, sequence, or single base pair

differences. In typical applications, only one signal is reported from a probe set, the remaining features serving as quality assurance and quality control indicators. For genomic fingerprinting, the variants related to the primary probe may also contain useful signals, and are also reported.

Figure 1:Overview of the Affymetrix microarray system.



APE : apurinic endonuclease; TDT : terminal deoxynucleotidyl transferase

2 Methods

2.1 Microarray platform selection

The two types of custom Affymetrix DNA microarray formats which could be used for genotyping are the Tiling Arrays and Resequencing Arrays. Choice of format in general governs the default reagent and protocols which can be accessed directly from Affymetrix. Probes on tiling arrays are spaced at a specified regular distances across a given genomic sequence (*e.g.* one probe every 500 bp). For resequencing arrays, the sequence for a region of interest is provided and four 25 base probes are designed for every base pair for the entire length of the sequence. Each member of a four probe set differs at the central (13th) bp at which an A, C, G or T will be incorporated. Probes are designed for both strands so that each base pair within the sequence is interrogated eight times.

Since the purpose of the multipathogen chip is to use probes derived from multiple source organisms, neither of the two standard custom designs fit our direct requirements. Through discussions with Affymetrix, a modified Tiling Array format was selected such that up to five probes would be provided for each sequence submitted for evaluation. In addition to control probes, whatever space remained on the array design would be filled with nonspecific probes selected from the existing Affymetrix probe library. The design contract with Affymetrix (executed as a subcontract with Canada West Biosciences) allows DRDC to retain ownership of our own probe designs, while using part of the Affymetrix probe library under licence.

2.2 Selection of targets

In principal, given the 25 base pair size of the oligonucleotide probes on the Affymetrix microarray, an ideal array could sample any possible sequence (known or unknown) if all possible 25-base oligonucleotides were spotted on the array. This would be A,C,G or T at all possible positions, which is 4^{25} oligonucleotide sequences, or ~1.126×10^{16} individual sequences. Current maximal capacity of the Affymetrix array system is approximately 1 million probes per array. It would require 1×10^{10} microarrays to cover most of the possible sequences. Thus, designing all possible 25 base pair sequences was not a practical nor an affordable approach.

Targeted probes are categorized as SNP (single nucleotide polymorphism) or non-SNP. SNP probes are included to better differentiate between strains of the same species. The target sequence submitted is 49 bp or less and contains one or more SNPs. For a sequence with one SNP, a set of 5 probes covering different segments of the target sequence is created for each of A, C, G and T at the variant base, resulting in a total of 20 probes. Thus, an organism with ‘A’ at the target site would register high intensity signal for the 5 ‘A’ probes and low intensity for the remaining 15 probes. For sequences containing more than one variant within a 49 base pair region, the number of probes increases accordingly. The mismatch SNP probe variants of a specific probe sequence (differing by one or a few bases from the specific probe), are used to assess non-specific or variant binding to probe sites and are useful for genomic fingerprinting.

Non-SNP probes have little sequence commonality and are used to differentiate at the species level. The target sequences are much longer than those used for SNP design and the probes, ranging from 1 to 15 unique sequences, can be spread over a large section of genomic DNA. Multiple target sequences (SNP and non-SNP) were submitted for each organism of interest to

ensure detection even if some probes failed to perform as expected. Ideally, all probes designed using a specific organism's DNA sequence should produce high intensity signal while the remaining probes (off-target) should have little to no signal.

In order to rationally develop a library of probes suitable for identifying the maximal number of agents, we focused on regional microvariation within sequenced genomes of interest. Sequences for probe design included those that encompassed regions that differed between strains of the same species, especially those from Category A bacteria. Also included were regions that were constant within a species but differed between species, virulence genes, and antibiotic resistance genes.

2.3 Target sequence extraction

The first step in identifying regions of interest was to review the existing literature on bacterial microarray genotyping and strain differentiation. This provided a partial list of genes to include in our search. Next, various online databases were investigated for genes of interest. Initially, the NCBI Protein Clusters database [5] was used. Protein Clusters provides curated and non-curated clusters of related protein reference sequences. The database was searched by species and the protein clusters of that species were targeted by the level of conservation. For example, strain variants in *Bacillus anthracis* were identified by selecting clusters conserved to the *Bacillus cereus* or *Bacillus anthracis* group level. Selecting a cluster of interest revealed the list of all strains included in the cluster. Variants within the sequence were then identified by viewing the detailed alignment. When variants were found, the DNA sequence was retrieved by clicking on the locus tag, then on the sequence viewer.

Antibiotic resistance genes were obtained from the Antibiotic Resistance Genes Database [6]. The majority of the sequences used for probe selection were obtained from VFDB, the Virulence Factors of Pathogenic Bacteria database [7]. This database provided FASTA formatted (plaintext for database searches) sequences of virulence genes and sequences that can be used for comparative genomics. Additional strains of interest for inclusion in the microarray design were provided by Dr Kingsley Amoako (Canadian Food Inspection Agency, Lethbridge, Alberta). In addition, the coding sequences for hypoxanthine guanine phosphoribosyltransferase (HPT) and adenine phosphoribosyltransferase (APRT) from multiple species of origin were included for future applications. Species and strains represented on the microarray are itemized in Annex A.

A master Excel file was created in order to manage the selected sequence segments. This file contained a number of data points: probe name, organism used to obtain the sequence, gene ID/Accession Number/Locus ID used to locate the gene, start and end base positions of the sequence used, length of the sequence segment used, first and last 8 bp of the selected sub-sequence, gene name and description, the strain the sequence was derived from, and the complete sequence segment. To determine which strains matched which sequence (beyond and including the sequence source strain), sequences were initially tested using nucleotide BLAST tool [8] against nucleotide reference sequences, then whole-genome shotgun sequences. For strains that differed by single base pair variations, single-nucleotide polymorphism (SNP) target sequences were prepared. These probe sequences were 49 bases in length with the variant, designated by an "n", in the 25th (central) position.

From this master file, two files were prepared for Affymetrix. The first was the instruction file listing the probe name, start and end positions of the probe sequence, first and last 8 bp of the

probe sequence and a description of the probe. The second file included all the probe sequences in FASTA format, each identified by the probe name provided in the instruction file. Once Affymetrix received the instruction and FASTA sequence files, five 25-mer probes were designed for each probe sequence submitted, using Affymetrix proprietary software. Degenerate or redundant probes were removed and a list of the proposed microarray design was returned for evaluation. The final microarray design was assembled using 81,678 probes from 11,516 unique microbial sequences, 24,660 probes from 264 SNP sequences, and approximately 140,000 non-specific probes along with controls to fill in the 220,678-probe chip. Annex A contains the listing of species and strain-specific represented, and how many probes that were used in the final design.

2.4 Microarray in silico verification

The straight text listing of oligonucleotide sequences printed onto the array was analyzed by testing all the sequences in the design against the entire NCBI non-redundant nucleotide database using iterative BLAST searches. PERL scripts were developed to run serial segments of the dataset (being too large to submit as a single set), and to log the returned sequence data, predicted species (and strain), and general annotations including accessions, of all hits against the submitted oligonucleotide sequences.

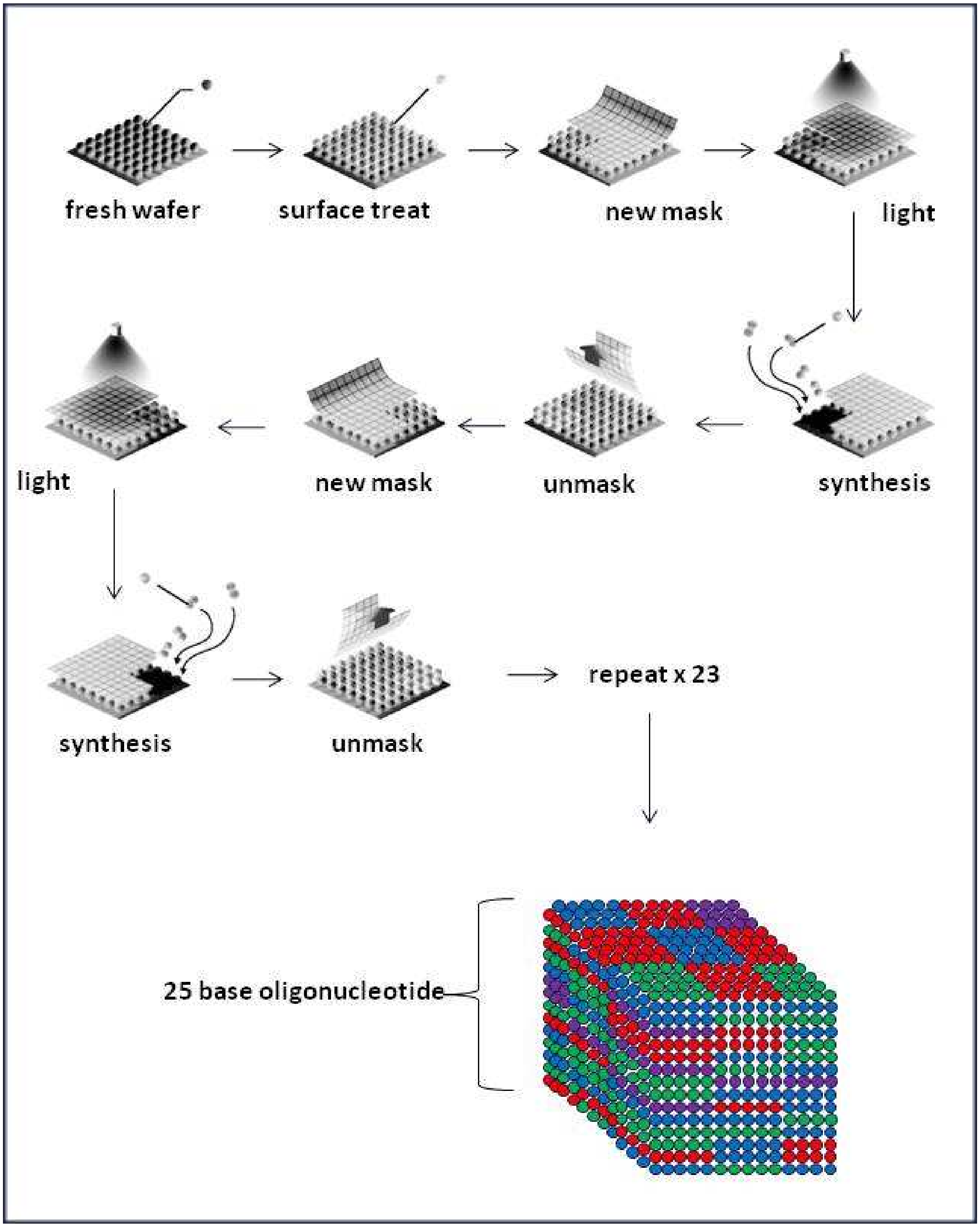
Since the entire feature sequence set was designed using publicly available databases, of which NCBI comprises a large, if not exhaustive aggregation, it was anticipated that *in silico* testing would recapitulate the designed species and strain identifications. It was also expected that due to sequence data errors and accession-specific variations within the database, that some sequences designed as unique probes (single species, single target) would actually align to accessions other than the record of origin.

2.5 Microarray fabrication

Affymetrix photolithography

The fabrication of the microarray per se is one key to the Affymetrix product package. Driven by the availability of photomicrolithography in microfabrication of microcircuitry, Affymetrix developed the method to template microarray chips using multiple lithographic overlays combined with photo-activatable oligonucleotide synthesis chemistry (Figure 2, adapted from Affymetrix Inc.). The technique allows for submicron precision in placement of oligonucleotide synthesis reactions on the surface of the silicon microarray wafer. Using multiple overlays, each site can be photoactivated differentially, and the different oligonucleotides synthesized stepwise. The more features on the array, the more overlays are required. Although photomicrolithography has been reported to produce some truncated probe sequences within each feature, the chip design includes truncation variants which can be used to verify signals from the features, or the absence of signal from the truncated probes as a set. This is generally done within the signal extraction software.

Figure 2: Photomicrolithography of microarrays. (adapted from Affymetrix Inc., Santa Clara, CA)



2.6 Microarray testing

Microbial DNA samples

Table 1 lists the microbial DNA samples that were used for preliminary testing of the custom microarray design. DNA samples from *E. coli* were prepared by the contractor, while DNA from level 2 and level 3 microbes were prepared by DRDC Suffield staff in the DRDC Suffield BSL2 or BSL3 labs respectively. BSL2 and BSL3 DNA extracts were tested for product sterility using standard procedures in the containment facility prior to release for microarray testing.

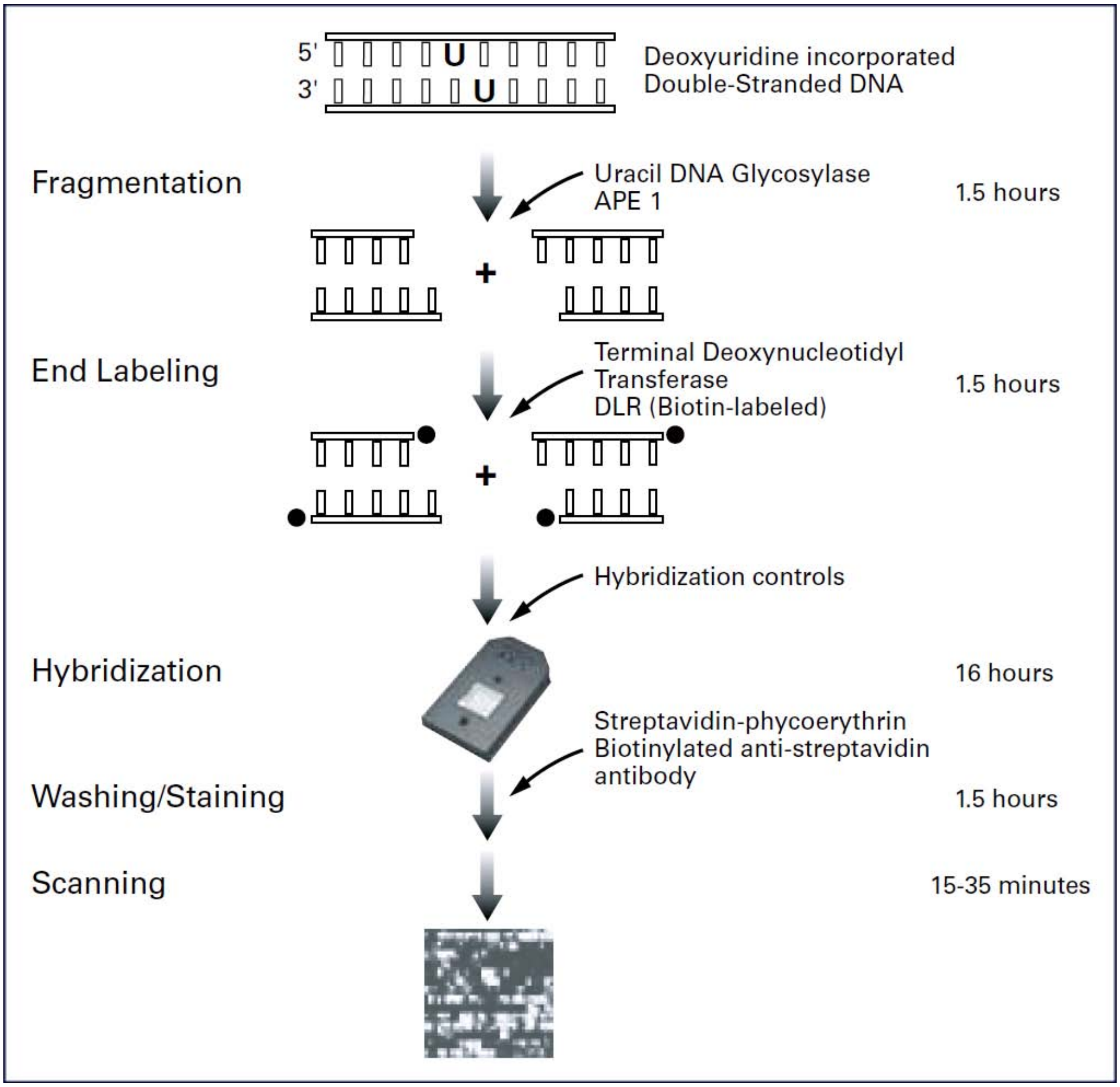
Table 1: DNA extracts used in initial testing of the microarray

Genus	Species	Strain / Isolate
Escherichia	***coli***	JM109
Bacillus	***anthracis***	RP42
Bacillus	***cereus***	ATCC 11778
Yersinia	***pestis***	19428
Yersinia	***enterocolitica***	

Affymetrix DNA labeling

The sample labeling method used in this study involved preparing a random-primed synthesis reaction incorporating uracil instead of thymine into the newly synthesized DNA using genomic DNA as the template, followed by direct end-labeling of the product DNA with biotinylated nucleotide, as shown in Figure 3. The detailed protocol used to label target DNA (per sample) is reproduced in Annex B:

Figure 3: End labelling sample DNA with terminal deoxynucleotide transferase (TdT).



2.7 Data reduction and analysis

Much of the data reduction work involved with microarrays involves feature extraction from the image to a spreadsheet, aligning the signals to the annotated target list, and data curation. These elements are contained within and managed by the Command Console software. Subsequent analysis involves normalization of data sets within the experimental series (between arrays), followed by comparison of test data to control data. In the case of genomic identification or fingerprinting, as on our array design, comparison to control arrays is not required for initial

assignment of genera and species. For the purposes of preliminary array design testing, no exhaustive comparison was undertaken.

Using tools developed for the open source microarray system (Chromablast) [9], data were reviewed without requiring prior normalization of signals. We compared data sets from *E. coli*, *B. anthracis*, *Y. pestis* and *Y. enterocolitica*, to determine qualitatively whether the target-specific array elements could discriminate between the samples. Data from the non-specific probe sets were not considered in this initial assessment. Student’s t tests were performed pairwise on data sets to determine whether this analysis was informative relative to the heat map display.

3 Results and Discussion

Data were obtained on the Affymetrix custom-designed microarray for DNA from *Escherichia coli*, *Yersinia pestis*, *Yersinia enterocolitica* and *Bacillus anthracis*. Although a number of analytical tools are available for comparing and estimating distance in genomic fingerprint data [10,11], for this verification of concept and function, qualitative comparison was sufficient.

Using Chromablast [9], a heat map representing relative signal values was developed for a series of technical replicates of *E. coli* used for the microarray testing. An excerpt showing a region of the resultant heat map is shown in Figure 4. Uniform heat map colour across the replciates would indicate perfect concordance between replicatees. The excerpted region shows some examples of this, as well as some replicates with varying colour, indicating some variation across the replicates. In Figure 4, green represents low value intensities (*i.e.* background to about 12% of maximum intensity, 0 to 6 in log base 2), and bright red indicates maximal intensity, as indicated in the scale below the heat map. The absolute scale of variation between non-normalized array data sets is thus seen to be about $\pm$ 30% within individual probe sets. This is verified by numerical analysis of the raw intensity data. Most of this variation is concentrated within the lower intensity values, where the standard deviation as a fraction of the mean is maximal. Above the mean signal intensity (~7.0 in log 2), the maximum signal variation per probe set is about $\pm$ 5% (Figure 5).

In practice, this suggests that a pruning of low-intensity signals may be useful to refine discrimination between samples versus knowns. Alternatively, a weighting factor could be applied to bias discriminatory decisions towards higher intensity signals. One method to compensate for signal variation between replicate arrays is to use the Student's t test to compare knowns to unknowns, or to detect outliers within the replication set for a known sample. In the case of the *E. coli* replicated data set, for the complete data set, including the lowest value probe intensities (15,533 probe sets), less than 2% of all signals in a pairwise comparison have a t-test value of less than 0.05. If only the signals greater than background are considered (9335 probe sets), the number of t-test values less than 0.05 falls to ~1% (72 probe sets). "Significant" t-test results obtained for low-intensity signals (low confidence) are removed by this strategy. Small occurrences of outlier or systematically unreliable signal sets, as indicated by this analysis, are unlikely to interfere with discrimination between different genera or species, but may complicate detailed discrimination between closely related strains.

An initial survey of *E. coli*, *B. anthracis*, *Y. pestis* and *Y. enterocolitica* on the microarray revealed that even at a high level view, the array could easily discriminate *E. coli* from *B. anthracis* (Figure 6-A,C). Note that these plots are of data not normalized post extraction, since the Affymetrix signal processing software applies in-process normalization using the internal controls. Comparison of the *Y. pestis* versus *E. coli* data suggested that the *Y. pestis* DNA sample contained DNA from E. *coli* or a related species.

Figure 4: Comparison of DNA from various E. coli replicates.
See text for detailed description.

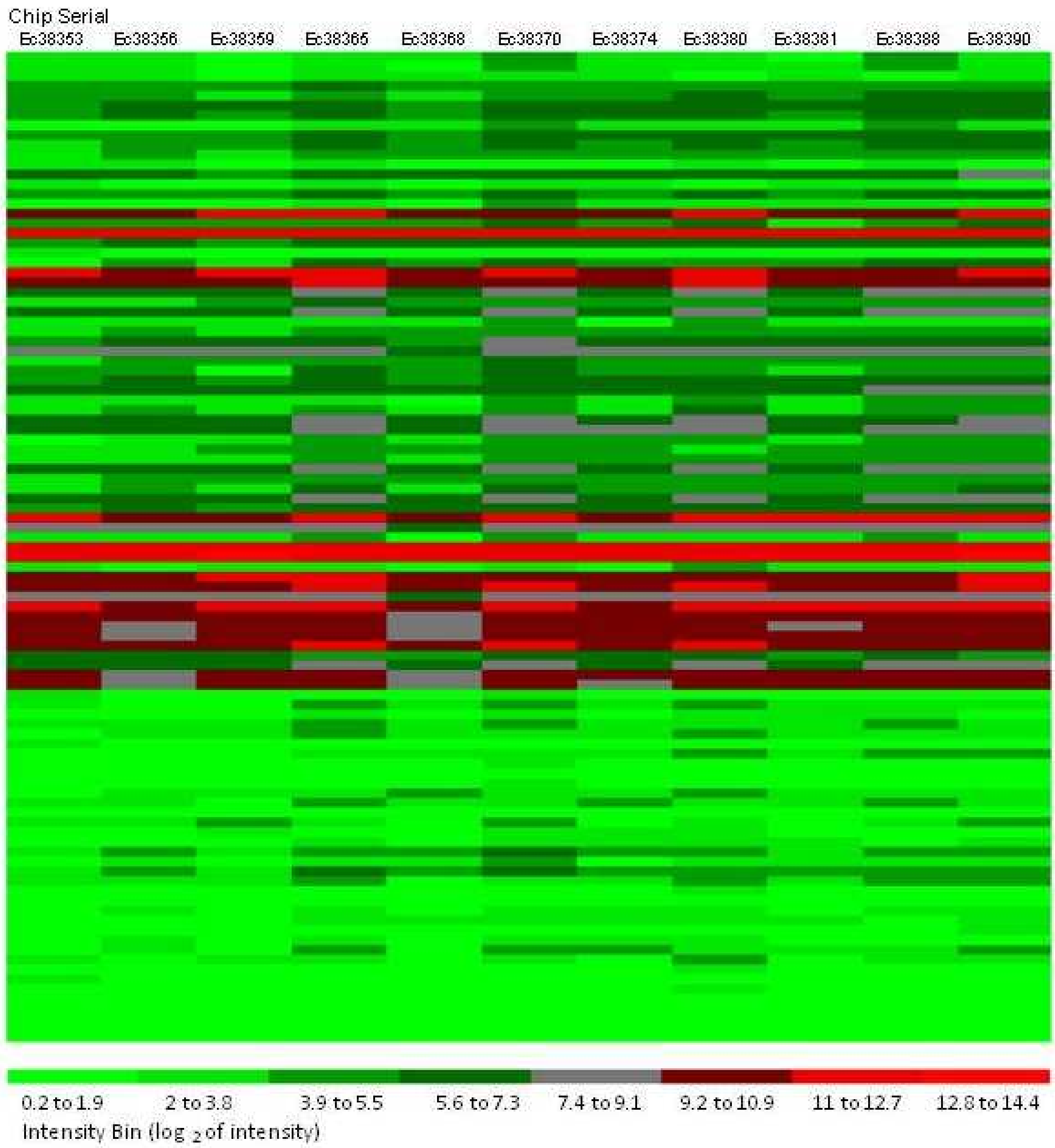
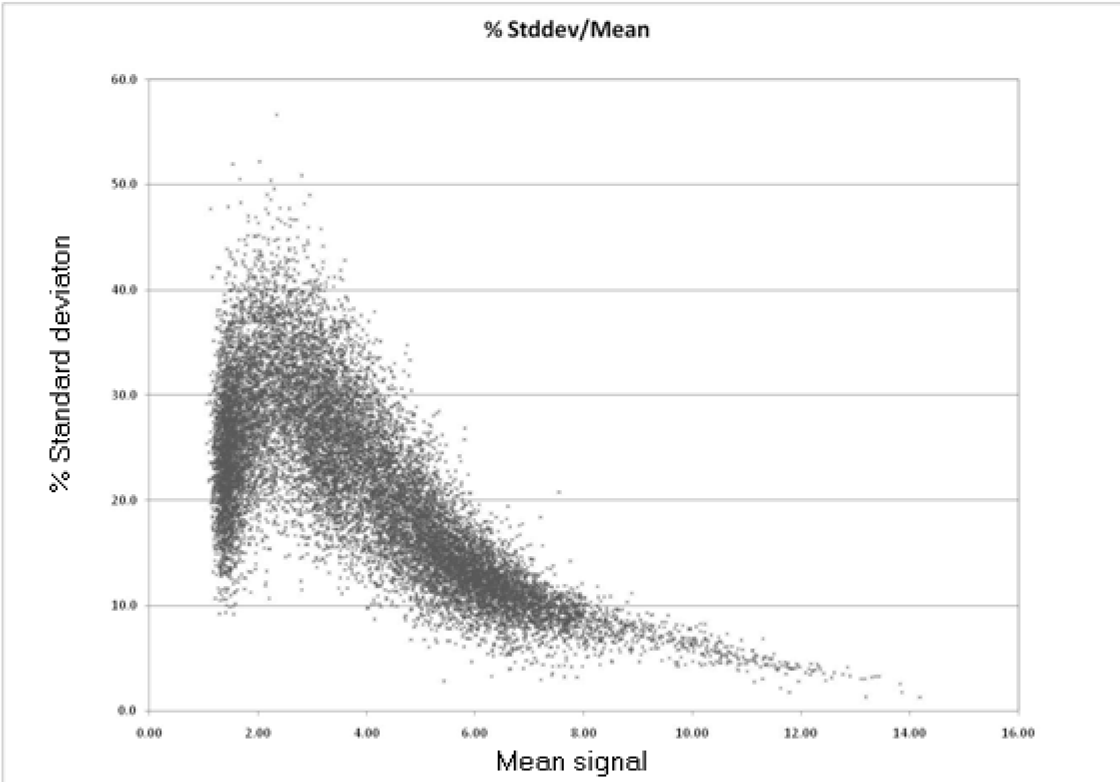
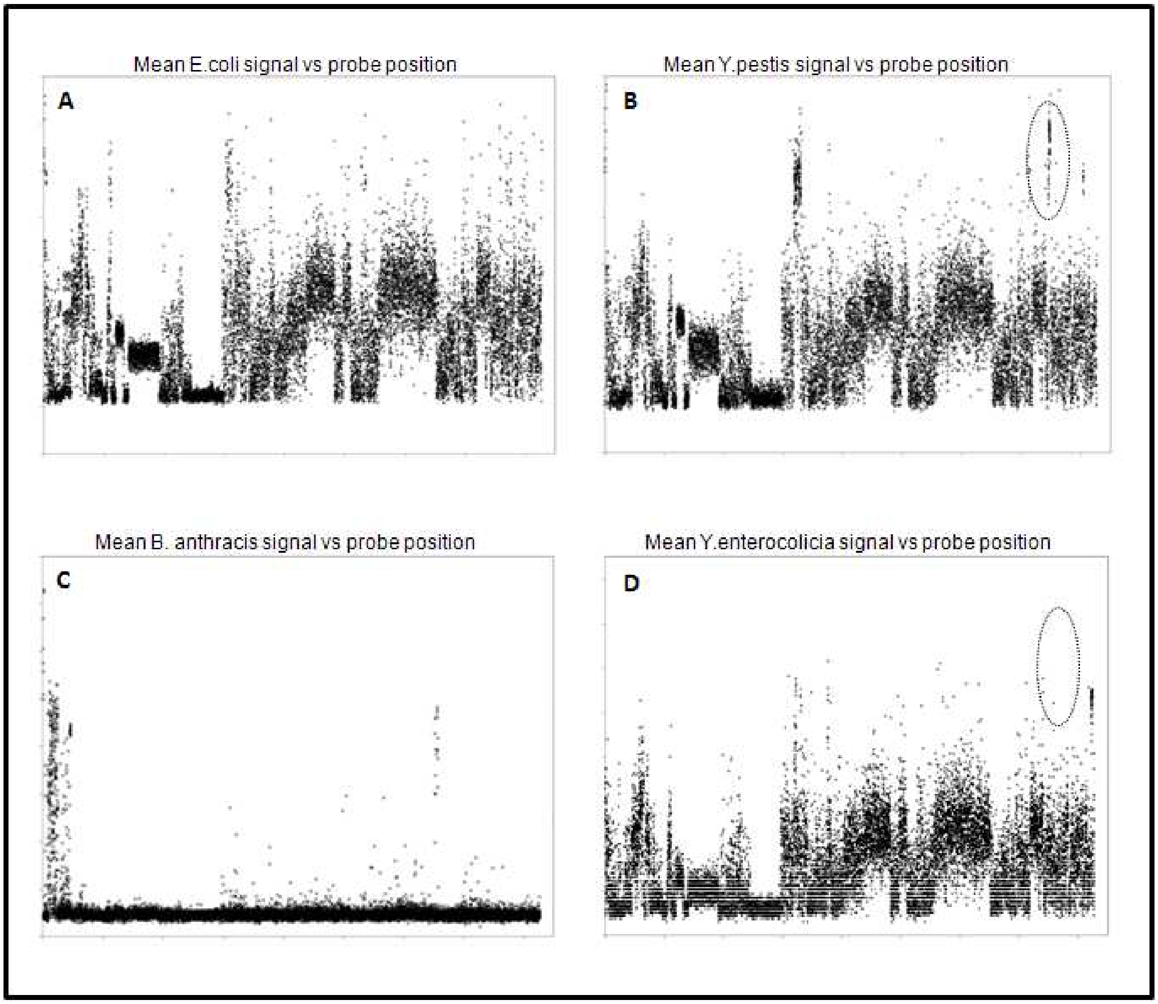


Figure 5: Standard deviation versus mean signal value.



Closer inspection of the plot in Figure 6-B showed the presence of signals not seen in the *E. coli* or the *Y. enterocolitica* samples (circled region in 6-B,D), indicating that this sample contained some unique DNA, and thus was either contaminated during preparation, or in the original culture stock. Upon detailed review of the signal data, it was apparent that even though the putative *Y. pestis* sample did indeed contain many signals similar to the *E. coli* pattern, a unique series of features designed to yield *Y. pestis*-specific signals, verified by in silico analysis, did indeed result in uniquely high signals for the *Y. pestis* data set, but not for any other data set. Our conclusion is that this sample did indeed contain *Y. pestis* DNA, but had suffered some contamination event either in original culture or in subsequent DNA preparation. Signals from both the intended DNA (*Y. pestis*) and from the contaminant were identified in this sample. In these plots, the *Y. enterocolitica* sample also appears to very like the *E. coli* sample, but differs noticeably from the *Y. pestis.*

Figure 6: Plots of signal intensity versus position.
See text for discussion.



4 Conclusions

The application of microarrays to microbial genotyping or fingerprinting is a technical compromise of time and difficulty versus data density. Single target or multiplex real-time PCR assays are faster and can be quantitative. Real-time PCR assays can in principle detect 2–4 targets per assay reaction, based on positive detection of specific sequences in known genomic targets. Routine PCR assays however, are not the best method of choice for detecting recombinants, variants, or the presence of non-target organisms. If an assay system could run hundreds of PCR reactions for each test sample, the analytical density of the microarray could be equaled. Typical microarray open source platforms can detect 20–50,000 targets per array, using a single labeling or amplification reaction. Open source microarray systems typically take 18–26 hours for a single execution, but each run encompasses the equivalent of 1–2 thousand multiplex PCR reactions.

Compared to PCR or gel electrophoresis assays, microarrays appear to be very expensive [13–15]. Microarray platforms are, for now at least, clear winners when the multiplex capabilities of the array systems are compared to comparable efforts on other platforms. Operating costs between open source microarray platforms and the Affymetrix system are similar, despite the higher hardware and consumables costs for the Affymetrix system. For example, the Affymetrix software package Command Console contains an integrated suite of tools for feature extraction, system quality assurance, and data curation. Required hands-on time for the feature extraction (from image to signal data on a spreadsheet) is measured in minutes, compared to the open source microarray system, where each array requires 1-3 hours of manual image data extraction. With the minimal hands-on time required for the post-hybridization, the automation features of the Affymetrix system represent its greatest operational advantage over the open source microarray platforms. Although initial costs are greater with the Affymetrix system, it seems likely that the cost differential will be very small once the accumulated savings in time and labour are considered.

The number of assays executed per microarray has the drawback that for some material sources, DNA from multiple species is likely to be present and may contribute to the measured signals [15]. If the microarray contains sufficient numbers of features and has a high degree of automation, endemic species are always going to give a signal, thus the mere presence of a signal of such a species in a given environmental or clinical context is not in itself meaningful [14-16]. Assays must be combined with other indices of suspicion (clinical signs, known exposure, suspect samples) in order to determine whether a given positive represents a real diagnosis or threat [1, 2, 17]. This is also true for most other molecular or microbiological assays currently in use. Simple detection of agent is insufficient to establish a diagnosis in a clinical setting.

In addition, as the sensitivity of assay systems improves (due to non-specific genomic amplification for example), out-of-context true positive signals (not within the normal range of endemics) may be detected [3,15,17]. Such signals may be due to sample contamination by workers, gratuitous sampling of infrequent (but locally intense) organism populations, or previously undetected genetic similarity between lab strains and endemic strains. Use of confirmatory assays of high-specificity (e.g. real-time PCR) will complement such data.

Given the requirement for technical expertise in operating a microarray system, and given the sensitivity to multiple targets in some samples, microarray systems will continue to require laboratory support. Microarray systems are in use in clinical centers, but point-of-care microarray systems are not imminent. On the other hand, time-to-result times are comparable to or better than conventional microbiology. Detailed testing of the current microarray and comparison to other microarray systems is underway. Additional testing with an expanded library of DNA samples and a wider sampling of species is required to fully assess the value of the microarray as a tool for diagnosis, detection, and identification of microbial samples.

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Annex A Species and strain-specific probes in the final array design.

Organism	Strain / details	Probes on Array
Acinetobacter baumannii	ACICU	38
Acinetobacter baumannii	ATCC 17978	54
Acinetobacter baumannii	AYE	143
Acinetobacter baumannii SNP	baumannii	20
Acinetobacter baumannii	baumannii	15
Acinetobacter baumannii	plasmid pSUN-5	5
Acinetobacter baumannii	SDF	44
Acinetobacter baumannii HPT	ATCC 17978	5
Acinetobacter baumannii HPT	AYE	5
Acinetobacter baumannii HPT	baumannii	5
Bacillus anthracis	Ames ancestor	140
Bacillus anthracis	Ames ancestor plasmid pX01	5
Bacillus anthracis	Ames ancestor plasmid pX02	25
Bacillus anthracis	anthracis	45
Bacillus anthracis	Australia 94	6
Bacillus anthracis	Kruger	5
Bacillus anthracis	Sterne	55
Bacillus anthracis APRT	A2012 plasmid pXO1	5
Bacillus anthracis APRT	Ames	5
Bacillus anthracis HPT	A0442	5
Bacillus anthracis HPT	anthracis	5
Bacillus anthracis HPT	anthracis	10
Bacillus anthracis plasmid	Sterne plasmid pX01+pX02-	10
Bacillus anthracis SNP	A2012	20
Bacillus anthracis SNP	anthracis	780
Bacillus anthracis SNP	other anthracis	200
Bacillus anthracis SNP	W. North America	20
Bacillus cereus	ATCC 10987	179
Bacillus cereus	ATCC 14579	200
Bacillus cereus	B. cereus plasmid pBCX01	5
Bacillus cereus	E33L	45
Bacillus cereus	G9241	5
Bacillus cereus group SNP	Bacillus	1800
Bacillus cereus HPT	E33L	5
Bacillus cereus SNP	ATCC 10987	80
Bacillus cereus SNP	ATCC 14579	320

Bacillus cereus SNP	B. cereus plasmid pBCXO1	320
Bacillus cereus/anthracis SNP	B. cereus plasmid pBCXO1	20
Bacillus amyloliquefaciens APRT	FZB42	5
Bacillus clausii	KSM-K16	5
Bacillus clausii APRT	KSM-K16	5
Bacillus halodurans	C-125	5
Bacillus halodurans APRT	C-125	5
Bacillus licheniformis	ATCC 14580	35
Bacillus licheniformis APRT	ATCC 14580	5
Bacillus pumilus APRT	SAFR-032	5
Bacillus subtilis	168	25
Bacillus subtilis APRT	168	5
Bacillus thuringiensis	97-27	115
Bacillus thuringiensis	Al Hakam	130
Bartonella bacilliformis	ATCC 35685	175
Bartonella henselae	Houston-1	270
Bartonella quintana	Toulouse	245
Bartonella tribocorum	CIP 105476	330
Bordetella SNP	Bordetella	20
Bordetella avium	197N	440
Bordetella avium APRT	197N	5
Bordetella bronchiseptica APRT	RB50	5
Bordetella bronchiseticas	RB50	75
Bordetella parapertussis	12822	268
Bordetella pertussis APRT	Tohama I	5
Bordetella pertussis	Bordetella	5
Bordetella pertussis	Tohama I	615
Bordetella petrii APRT	DSM 12804	5
Borrelia afzelii APRT	PKo	5
Brucella SNP	9-941	20
Brucella	all brucella	250
Brucella HPT	all brucella	5
Brucella abortus	9-941	30
Brucella abortus	S19	45
Brucella abortus SNP	melitensis/abortus	40
Brucella abortus APRT	9-941	5
Brucella abortus/melitensis SNP	abortus/melitensis	20
Brucella abortus/suis SNP	abortus/suis	20
Brucella canis	ATCC 23365	15
Brucella canis	S19	5
Brucella canis HPT	ATCC 23365	10
Brucella melitensis	16M	427
Brucella melitensis	2308 bv Abortus	210
Brucella melitensis	bv Melitensis	10
Brucella melitensis	bv Suis 686	5
Brucella ovis	ATCC 25840	82

Brucella ovis	bv Abortus 2308	35
Brucella suis	1330	25
Brucella suis	ATCC 23445	5
Brucella suis	ATCC 23447	5
Brucella suis	ATCC 25840	10
Brucella suis	bv. 4 str. 40	15
Brucella suis/abortus SNP	suis/abortus	80
Burkholderia SNP	Burkholderia	1160
Burkholderia HPT	all burkholderia	5
Burkholderia mallei APRT	ATCC 23344	5
Burkholderia mallei	ATCC 23344	40
Burkholderia mallei	PRL-20	5
Burkholderia multivorans APRT	ATCC 17616	5
Burkholderia pseudo/mallei SNP	pseudomallei/mallei	20
Burkholderia pseudo/mallei	Burkholderia	15
Burkholderia pseudomallei	668	10
Burkholderia pseudomallei	1710b	5
Burkholderia pseudomallei	392f	5
Burkholderia pseudomallei SNP	B7210	40
Burkholderia pseudomallei	K96243	75
Burkholderia pseudomallei SNP	pseudomallei	340
Burkholderia pseudomallei	pseudomallei	5
Burkholderia pseudomallei	T18-1984	5
Burkholderia pseudomallei HPT	91	5
Burkholderia pseudomallei HPT	668	5
Burkholderia pseudomallei HPT	NCTC 13177	5
Burkholderia pseudomallei APRT	668	5
Burkholderia thailandensis APRT	E264	5
Campylobacter concisus APRT	13826	5
Campylobacter fetus	82-40	440
Campylobacter hominis APRT	ATCC BAA-381	5
Campylobacter jejuni APRT	doylei 269.97	5
Campylobacter jejuni	269.97 ss doylei	476
Campylobacter jejuni	81116 (NCTC 11828)	351
Campylobacter jejuni	81-176	349
Campylobacter jejuni	jejuni	60
Campylobacter jejuni	NCTC 11168	560
Campylobacter jejuni	plasmid pCjA13 t	5
Campylobacter jejuni	RM 1221	304
Campylobacter jejuni APRT	81-176	5
Campylobacter jejuni plasmid	81-176 plasmid pVir	5
Chaetomium atrobrunneum	atrobrunneum	5
Chaetomium funicola	funicola	29
Chaetomium funicola	OC13	5
Chaetomium funicola	olrim130	5
Chaetomium thermophilum	CT2	20

Chaetomium thermophilum	MTCC 6350	5
Chaetomium thermophilum	thermophilum	85
Chlamydia abortus	S26/3	115
Chlamydia caviae	GPIC	115
Chlamydia felis	Fe/C-56	120
Chlamydia muridarum	Nigg (MoPn)	115
Chlamydia pneumoniae	AR39	115
Chlamydia pneumoniae	CWL 029	5
Chlamydia trachomatis	D/UW-3/CX	175
Chlamydia trachomatis	HAR-13	15
Chlamydia trachomatis	trachomatis	5
Clostridium botulinum APRT	Alaska E43	10
Clostridium botulinum APRT	ATCC 3502	5
Clostridium botulinum APRT	Eklund 17B	5
Clostridium botulinum APRT	Okra	5
Clostridium botulinum	A str. ATCC 19397	5
Clostridium botulinum	ATCC 3502	40
Clostridium botulinum	B str. Eklund 17B	5
Clostridium botulinum SNP	B1 str. Okra plasmid pCLD	20
Clostridium botulinum	B1 str. Okra plasmid pCLD	5
Clostridium botulinum	Bf	5
Clostridium botulinum SNP	botulinum	1860
Clostridium botulinum	C str. Eklund	5
Clostridium botulinum SNP	C. botulinum A strains	100
Clostridium botulinum	C. botulinum A strains	5
Clostridium botulinum	Clostridium botulinum	15
Clostridium botulinum	Hall 183	5
Clostridium botulinum HPT	Alaska E43	15
Clostridium botulinum HPT	Eklund 17B	10
Clostridium botulinum HPT	Loch Maree	20
Clostridium botulinum HPT	Okra	5
Clostridium botulinum	A3 str. Loch Maree	5
Clostridium acetobutylicum	ATCC 824	25
Clostridium beijerinckii	NCIMB 8052	20
Clostridium difficile	630	45
Clostridium difficile		15
Clostridium difficile HPT	difficile	5
Clostridium kluyveri APRT	DSM 555	5
Clostridium novyi	ATCC19402	45
Clostridium novyi	NT	40
Clostridium perfringens APRT	SM101	5
Clostridium perfringens	13	111
Clostridium perfringens	ATCC 13124	66
Clostridium perfringensS		20
Clostridium perfringens	SM101	65
Clostridium perfringens HPT	13	5

Clostridium perfringens HPT	ATCC 13124	10
Clostridium perfringens HPT	SM101	10
Clostridium perfringens plasmid	plasmid pCP13	5
Clostridium tetani	E88	55
Clostridium tetani HPT	tetani	5
Clostridium thermocellum	ATCC 27405	15
Corynebacterium diphtheriae	diptheriae	5
Corynebacterium diphtheriae	NCTC 13129	165
Corynebacterium efficiens	YS-314	65
Corynebacterium glutamicum	ATCC 13032	20
Corynebacterium glutamicum	R	69
Corynebacterium glutamicum APRT	ATCC 13032	5
Corynebacterium jeikeium	K411	110
Coxiella burnetii	CbuG Q212	15
Coxiella burnetii	Dugway 5J108-111	25
Coxiella burnetii	MSU Goat Q117	29
Coxiella burnetii	RSA 331	15
Coxiella burnetii	RSA 334	5
Coxiella burnetii	RSA 493	178
Coxiella burnetii HPT	Dugway	5
Coxiella burnetii HPT	burnetti	10
Enterococcus faecalis	faecalis	5
Enterococcus faecalis	MMH594	5
Enterococcus faecalis	V583	145
Enterococcus faecalis APRT	V583	5
Enterococcus faecalis HPT	faecalis	5
Escherichia coli	536	1035
Escherichia coli	1226	5
Escherichia coli	1334	5
Escherichia coli	55989	20
Escherichia coli	042	70
Escherichia coli	17-2	25
Escherichia coli	536 (UPEC)	30
Escherichia coli	B171	85
Escherichia coli	C1845	5
Escherichia coli	CFT 073 (UPEC)	516
Escherichia coli	coli	182
Escherichia coli	coli/shigella	5
Escherichia coli	E. coli plasmid pC15-1a_016	5
Escherichia coli	E/99 3-2 SHV	10
Escherichia coli	E2348/69	285
Escherichia coli	E45035	5
Escherichia coli	EC7372	5
Escherichia coli	EU2657	5
Escherichia coli	EU4855 plasmid	5
Escherichia coli	H11128	25

Escherichia coli	H11129	5
Escherichia coli	K12	38
Escherichia coli	K12 substr. MG1655	25
Escherichia coli	K983802	5
Escherichia coli	KS52	5
Escherichia coli	O157:H7 EDL933	345
Escherichia coli	plasmid	15
Escherichia coli	plasmid p541	5
Escherichia coli	plasmid pEC365	5
Escherichia coli	plasmid pGR2439	5
Escherichia coli	plasmid pMEL2	3
Escherichia coli	plasmid RZA92	5
Escherichia coli	Sakai(EHEC O157:H7)	11
Escherichia coli	SMS-3-5	20
Escherichia coli	Str. 01 (APEC)	50
Escherichia coli	Toho-1	5
Escherichia coli	UTI89 (UPEC)	65
Escherichia coli	YMC02/08/U310	5
Escherichia coli	SMS-3-5	5
Escherichia coli APRT	O157:H7 EDL933	5
Escherichia coli HPT	ATCC 8739	5
Escherichia coli HPT	E24377A	5
Escherichia coli HPT	F11	4
Escherichia coli HPT	HS	5
Escherichia coli plasmid	plasmid pAPEC-O1-ColBM	40
Escherichia coli strain EO 516	EO 516	5
Francisella holarctica APRT	OSU18	5
Francisella holartica	FTNF002-00	15
Francisella holartica	holartica	31
Francisella holartica	LVS	35
Francisella holartica	OSU18	25
Francisella holartica HPT	holartica	10
Francisella holartica SNP	FSC022	40
Francisella holartica SNP	FTNF002-00	80
Francisella holartica SNP	HOL 257	20
Francisella holartica SNP	holartica	240
Francisella holartica SNP	LVS	20
Francisella holartica SNP	OSU18	100
Francisella novicida	U112	105
Francisella novicida HPT	U112	5
Francisella novicida SNP	GA99-3548	700
Francisella novicida SNP	novicida	7480
Francisella novicida SNP	U112	620
Francisella tularensis	ATCC 6223	46
Francisella tularensis	francisella	5
Francisella tularensis	fsc033	5

Francisella tularensis	FSC198	15
Francisella tularensis	plasmid pOM1	5
Francisella tularensis	SCHU S4	411
Francisella tularensis	tularensis	52
Francisella tularensis	WY96-3418	55
Francisella tularensis SNP	SCHU	180
Francisella tularensis SNP	tularensis	580
Francisella tularensis SNP	WY96	100
Francisella tularensis SNP	WY96-3418	20
Francisella	Francisella	15
Francisella holartica/novicida	holartica/novicida	5
Francisella holartica/tularensis	holartica/tularensis	25
Francisella novicida/tularensis	novicida/tularensis	30
Francisella tularensis/holartica SNP	tularensis/holartica	20
Haemophilus ducreyi	35000 HP	405
Haemophilus influenzae APRT	86-028NP	5
Haemophilus influenzae APRT	Rd KW20	5
Haemophilus influenzae	12	30
Haemophilus influenzae	1007	89
Haemophilus influenzae	3179B	5
Haemophilus influenzae	86-028NP	336
Haemophilus influenzae	AM30	25
Haemophilus influenzae	C54	5
Haemophilus influenzae	influenzae	5
Haemophilus influenzae	N187	5
Haemophilus influenzae	Pitt EE	275
Haemophilus influenzae	Pitt GG	299
Haemophilus influenzae	Rd	95
Haemophilus influenzae	Rd KW20	375
Haemophilus somnus	2336	205
Haemophilus somnus	129 PT	380
Helicobacter acinonychis	Sheeba	279
Helicobacter hepaticus	ATCC 51449	250
Helicobacter pylori APRT	J99	5
Helicobacter pylori	26695	438
Helicobacter pylori	HPAG1	377
Helicobacter pylori	J99	484
Human	Human	100
Klebsiella pneumonia APRT	MGH 78578	5
Lactobacillus delbrueckii APRT	subsp. bulgaricus ATCC 11842	5
Legionella pneumonphila	Philadelphia 1	793
Legionella pneumophila HPT	Corby	3
Legionella pneumophila HPT	Lens	5
Legionella pneumophila HPT	Paris	10
Legionella pneumophila HPT	Philadelphia 1	5
Legionella pneumophila	Corby	296

Legionella pneumophila	Lens	378
Legionella pneumophila	Paris	399
Legionella pneumophila	pneumophila	5
Listeria innocua	Clip 11262	105
Listeria ivanoviil	ATCC 19119	5
Listeria monocytogenes	monocytogenes	10
Listeria monocytogenes APRT	EGD-e	5
Listeria monocytogenes HPT	4b 2365	10
Listeria monocytogenes HPT	EGD-e	5
Listeria monocytogenes	4b 2365	260
Listeria monocytogenes	EGD-e sv 1/2A	453
Listeria monocytogenes	F2365	95
Listeria monocytogenes APRT	F2365	5
Listeria monocytogenes SNP	J1-194	1280
Listeria monocytogenes SNP	J2-064	80
Listeria monocytogenes	J2-064	5
Listeria monocytogenes SNP	monocytogenes	5180
Listeria welshimeri APRT	SLCC 5334	5
Listeria welshimeri	SLCC 5334	100
Mycobacterium avium APRT	K-10 ss paratuberculosis	5
Mycobacterium avium	104	263
Mycobacterium avium	K-10 ss paratuberculosis	743
Mycobacterium bovis APRT	BCG str. Pasteur 1173P2	5
Mycobacterium bovis	AF2122/97	15
Mycobacterium bovis	BCG Pasteur 1173P2	15
Mycobacterium gilvums	PYR-GCK	619
Mycobacterium leprae APRT	TN	5
Mycobacterium leprae	TN	379
Mycobacterium marinum APRT	M	5
Mycobacterium smegmatis	MC2155	543
Mycobacterium tuberculosis APRT	CDC 1551	5
Mycobacterium tuberculosis	CDC 1551	120
Mycobacterium tuberculosis	F11	15
Mycobacterium tuberculosis	H37 Ra	5
Mycobacterium tuberculosis	H37 Rv	682
Mycobacterium tuberculosis	tuberculosis/bovis	5
Mycobacterium ulcerans	Agy 99	504
Mycobacterium ulcerans APRT	Agy99	5
Mycobacterium ulcerans plasmid	Agy99 plasmid pMUM001	20
Mycobacterium van baalenii	PYR-1	702
Mycobacteriums sp.	JLS	650
Mycobacteriums sp.	KMS	120
Mycobacteriums sp.	MCS	45
Mycoplasma agalactiae	PG2	45
Mycoplasma capricolum	ATCC 27343	10
Mycoplasma gallisepticum	R	230

Mycoplasma genitalium	G37	50
Mycoplasma hyopneumoniae APRT	7448	4
Mycoplasma hyopneumoniae APRT	J	7
Mycoplasma hyopneumoniae	232	70
Mycoplasma hyopneumoniae	7448	35
Mycoplasma hyopneumoniae	J	30
Mycoplasma mobile	163K	105
Mycoplasma mycoides APRT	PG1	5
Mycoplasma mycoides	PG1	90
Mycoplasma penetrans	HF-2	250
Mycoplasma pneumoniae APRT	M129	5
Mycoplasma pneumoniae	M129	50
Mycoplasma pneumoniae	pneumoniae	5
Mycoplasma pulmonis APRT	UAB CTIP	5
Mycoplasma pulmonis	UABCTIP	74
Mycoplasma synoviae	53	10
Neisseria gonorrhoeae	FA 1090	205
Neisseria meningitidis	FAM18	188
Neisseria meningitidis	MC58	274
Neisseria meningitidis	neisseria	5
Neisseria meningitidis	str. 053442	164
Neisseria meningitidis	Z2491	281
Plasmid pBC16	Plasmid pBC16	5
Plasmid pLS1	Plasmid pLS1	5
Pseudomonas aeruginosa HPT	2192 Paer2_01_70	5
Pseudomonas aeruginosa HPT	PA01	10
Pseudomonas aeruginosa HPT	PA7	5
Pseudomonas aeruginosa	aeruginosa	5
Pseudomonas aeruginosa	PA01	1274
Pseudomonas aeruginosa	PA7	1015
Pseudomonas aeruginosa	UCBPP-PA14	317
Pseudomonas entomophila HPT	L48	5
Pseudomonas entomophila	L48	558
Pseudomonas fluorescens HPT	Pf-5	5
Pseudomonas fluorescens HPT	PfO-1	5
Pseudomonas fluorescens	Pf-5	710
Pseudomonas fluorescens	PfO-1	590
Pseudomonas mendocina HPT	ymp	5
Pseudomonas mendocina	ymp	645
Pseudomonas putida APRT	KT 2440	5
Pseudomonas putida HPT	GB-1	5
Pseudomonas putida HPT	KT 2440	5
Pseudomonas putida	F1	430
Pseudomonas putida	GB-1	607
Pseudomonas putida	KT 2440	706
Pseudomonas putida	W619	560

Pseudomonas stutzeri	A1501	480
Pseudomonas stutzeri HPT	A1501	5
Pseudomonas syringae APRT	pv. phaseolicola 1448A	5
Pseudomonas syringae	1448A	1042
Pseudomonas syringae	B728a	1021
Pseudomonas syringae	DC3000	1214
Pseudomonas syringae HPT	pv. phaseolicola 1448A	7
Pseudomonas syringae HPT	pv. syringae B728a	5
Pseudomonas syringae HPT	pv. tomato str. DC3000	5
Pseudomonas syringae plasmid	1448A large plasmid	50
Pseudomonas syringae plasmid	plasmid pDC3000A	20
Ricinus communis	communis	20
Rickettsia prowazekii	Madrid E	55
Rickettsia prowazekii	prowazekii	5
Rickettsia rickettsii	Iowa	70
Rickettsia rickettsii SNP	rickettsiae	60
Rickettsia rickettsii	rickettsii/africae/sibirica	5
Rickettsia typhi	Wilmington	55
Salmonella enterica APRT	Typhi str. CT18	5
Salmonella enterica	ATCC 9150 sv paratyphi A	168
Salmonella enterica	CT18	332
Salmonella enterica	enterica	5
Salmonella enterica	LT2	520
Salmonella enterica	RSK 2980 ss arizona sv 62	544
Salmonella enterica	SC-B67 sv Choleraesuis	201
Salmonella enterica	SPB7 sv Paratyphi B	207
Salmonella enterica	sv typhimurium	239
Salmonella enterica	Ty2	10
Salmonella enterica plasmid	pSN254	125
Salmonella enterica plasmid	SC-B67 plasmid pSCV50	10
Salmonella typhimurium	LT2	253
Salmonella typhimurium plasmid	LT2 plasmid pSLT	5
Shigella dysenteriae	plasmid pmK105	5
Shigella boydii	227	43
Shigella boydii	0-1392	20
Shigella boydii	CDC 3083-94	93
Shigella boydii	Sb227	300
Shigella boydii HPT	boydii	5
Shigella boydii plasmid	plasmid pSB4_227	15
Shigella dysenteriae APRT	Sd197	5
Shigella dysenteriae	197	107
Shigella dysenteriae	Sd197	130
Shigella dysenteriae plasmid	plasmid pSD1_197	171
Shigella flexneri	301	866
Shigella flexneri	8401	60
Shigella flexneri	2457T	80

Shigella flexneri	flexneri	45
Shigella flexneri	M90T	248
Shigella flexneri	multiple species	5
shigella flexneri HPT	flexneri	5
Shigella flexneri plasmid	M90T plasmid pWR501	15
Shigella flexneri plasmid	plasmid pPCP301	35
Shigella sonnei	Ss046	66
Shigella sonnei plasmid	str. 046 plasmid pSS_046	15
Staphylococcus aureus APRT	N315	5
Staphylococcus aureus HPT	aureus	5
Staphylococcus aureus	aureus	45
Staphylococcus aureus	COL	140
Staphylococcus aureus	JH1	15
Staphylococcus aureus	JH9	15
Staphylococcus aureus	MRSA 252	255
Staphylococcus aureus	MSSA 476	2
Staphylococcus aureus	Mu3	10
Staphylococcus aureus	Mu50	140
Staphylococcus aureus	MW2	350
Staphylococcus aureus	N315	20
Staphylococcus aureus	NCTC 8325	29
Staphylococcus aureus	Newman	15
Staphylococcus aureus	RF122	203
Staphylococcus aureus	USA 300_TCH 1516	10
Staphylococcus aureus	USA 3000	27
Staphylococcus epidermidis APRT	RP62A	5
Staphylococcus epidermidis	ATCC 12228	62
Staphylococcus epidermidis	RP62A	60
Staphylococcus epidermidis HPT	epideridis	5
Staphylococcus haemolyticus	JCSC 1435	80
Staphylococcus haemolyticus HPT	haemolyticus	5
Staphylococcus saprophyticus HPT	saprophyticus	5
Staphylococcus saprophyticus	ATCC 15305	95
Streptococcus agalactiae APRT	A909	5
Streptococcus agalactiae	2603 V/R	145
Streptococcus agalactiae	A909	200
Streptococcus agalactiae	agalactiae	5
Streptococcus agalactiae	FM027022	5
Streptococcus agalactiae	NEM316	75
Streptococcus agalactiae HPT	agalactiae	5
Streptococcus agalactiae HPT	CJB111	10
Streptococcus gordonii	Challis	150
Streptococcus mutans	UA 159	135
Streptococcus pneumoniae APRT	Hungary 19A-6	5
Streptococcus pneumoniae APRT	R6	5
Streptococcus pneumoniae HPT	Hungary 19A-6	6

Streptococcus pneumoniae HPT	pneumoniae	5
Streptococcus pneumoniae HPT	TIGR4	2
Streptococcus pneumoniae	CGSP14	87
Streptococcus pneumoniae	D39	154
Streptococcus pneumoniae	Hungary 19A-6	130
Streptococcus pneumoniae	pneumoniae	5
Streptococcus pneumoniae	R6	5
Streptococcus pneumoniae	TIGR4	185
Streptococcus pyogenes APRT	M1 GAS	5
Streptococcus pyogenes	Manfredo st M5	50
Streptococcus pyogenes	MGAS 10270 st M2	95
Streptococcus pyogenes	MGAS 10394 st M6	95
Streptococcus pyogenes	MGAS 10750 st M4	83
Streptococcus pyogenes	MGAS 2096 st M12	57
Streptococcus pyogenes	MGAS 315 st M3	85
Streptococcus pyogenes	MGAS 5005 st M1	35
Streptococcus pyogenes	MGAS 6180 st M28	80
Streptococcus pyogenes	MGAS 8232 st M18	65
Streptococcus pyogenes	MGAS 9429 st M12	10
Streptococcus pyogenes	pyogenes	5
Streptococcus pyogenes	SF370	150
Streptococcus pyogenes	SSI-1 st M3	36
Streptococcus pyogenes HPT	MGAS 10394	5
Streptococcus pyogenes HPT	MGAS 10750	5
Streptococcus pyogenes HPT	MGAS 8232	5
Streptococcus pyogenes HPT	pyogenes	5
Streptococcus sanguinis	SK36	232
Streptococcus sanguinis HPT	sanguinis	5
Streptococcus suis	05ZYH33	138
Streptococcus suis	98 HAH33	65
Streptococcus thermophilus HPT	LMG 18311	4
Streptococcus thermophilus	CNRZ1066	117
Streptococcus thermophilus	LMD-9	150
Streptococcus thermophilus	LMG 18311	135
Streptococcus thermophilus HPT	thermophilus	5
Treponema pallidum	Nichols	5
Treponema pallidum	pallidum	5
Treponema pallidum	SS14	30
Ureaplasma parvum APRT	ATCC 27815	5
Vibrio cholerae APRT	O395	5
Vibrio cholerae HPT	623-39	5
Vibrio cholerae HPT	RC385	5
Vibrio cholerae	1587	5
Vibrio cholerae	623-39	10
Vibrio cholerae	all other Vibrio cholerae	60
Vibrio cholerae	cholerae	5

Vibrio cholerae	MAK 757	5
Vibrio cholerae	MZO-2	5
Vibrio cholerae	MZO-3	5
Vibrio cholerae	N16961	1144
Vibrio cholerae	NCTC 8457	5
vibrio cholerae	O395	145
Vibrio cholerae	plasmid pTLC -1	5
Vibrio cholerae	plasmid pTLC -2	5
Vibrio cholerae	plasmid pTLC -3	5
Vibrio cholerae	plasmid pTLC -4	5
Vibrio cholerae	plasmid pTLC -5	5
Vibrio cholerae	RC385	5
Vibrio cholerae	V51	10
Vibrio cholerae HPT	cholerae	5
Vibrio cholerae HPT	V51	5
Vibrio fischeri	ES114	554
Vibrio parahaemolyticus	AQ3810	5
Vibrio parahaemolyticus	parahaemolyticus	5
Vibrio parahaemolyticus	RIMD 2210633	830
Vibrio vulnificus	CMCP6	764
Vibrio vulnificus	Vibrio vulnificus	5
Vibrio vulnificus	YJ016	443
Xanthomonas axonopodis APRT	pv. citri str. 306	5
Yersinia enterocolitica	8081	560
Yersinia enterocolitica	84-50	5
Yersinia enterocolitica	A127	177
Yersinia enterocolitica	W1024	10
Yersinia enterocolitica APRT	8081	5
Yersinia enterocolitica HPT	8081	10
Yersinia enterocolitica plasmid	8081 plasmid pYVe8081	94
Yersinia pestis	91001 bv Microtus	20
Yersinia pestis	Angola	38
Yersinia pestis	Antiqua	50
Yersinia pestis	bv Microtus str. 91001	15
Yersinia pestis	CO92	614
Yersinia pestis	KIM	65
Yersinia pestis	Nepal 516	20
Yersinia pestis	Pestoides F	15
Yersinia pestis	Y. pestis	5
Yersinia pestis APRT	Angola	5
Yersinia pestis APRT	CO92	5
Yersinia pestis APRT	KIM	5
Yersinia pestis HPT	CO92	10
Yersinia pestis plasmid	pIP1202	90
Yersinia pestis plasmid	91001 bv Microtus plasmid pCD1	10
Yersinia pestis plasmid	Angola plasmid pCD	5

Yersinia pestis plasmid	Pestoides F plasmid pCD	13
Yersinia pseudotuberculosis	IP 31758	115
Yersinia pseudotuberculosis	IP 32953	68
Yersinia pseudotuberculosis	pseudotuberculosis	5
Yersinia pseudotuberculosis	YP111	56
Yersinia pseudotuberculosis HPT	PB1/+	10
Yersinia pseudotuberculosis plasmid	IP32953 plasmid YV	12
Yersinia pseudotuberculosis plasmid	plasmid pYps IP31758.1	195
Yersinia pseudotuberculosis plasmid	plasmid pYps IP31758.2	45
Yersinia pestis/pseudotuberculosis	pestis/pseudotuberculosis	10
Yersinia pestis/pseudotuberculosis SNP	IP 31758	20
Yersinia pestis/pseudotuberculosis SNP	pestis/pseudotuberculosis	520

Annex B Detailed Protocols for microarray labeling

dUTP Incorporation - Using ROCHE Random Priming kit (no amplification)

1. Add 5 µg sample DNA (16 µl volume) to PCR tube. Incubate at 95 °C for 10 min.
2. During the above incubation, prepare the following (Incorporation mix):

   0.8 µl dH_2O
   0.8 µl of 1 mM dUTP
   1.6 µl of 0.5 mM dTTP
   2.0 µl of 0.5 mM dATP
   2.0 µl of 0.5 mM dCTP
   2.0 µl of 0.5 mM dGTP

3. Vortex the mixture briefly.
4. When the incubation in step 1 is almost finished, add the following to the incorporation mix:

   2.0 µl of the hexamer primer reaction mix
   1.0 µl of Klenow enzyme (keep on ice until use)

5. Vortex the mixture briefly and keep on ice until use.
6. Add 12.2 µl (total volume of the incorportation mixture) to the cooled sample DNA from step 1. Vortex briefly.
7. Incubate the sample at 37 °C for 2 hrs (program AFFY1).
8. After 2 hrs, take sample from incubator and add sufficient RNase-free H_20 to make a final volume of 28.2 µl (if required).
9. Incubate sample tube at 95 °C for 10 min

Fragmentation - Using AFFY GeneChip WT Terminal Labelling Kit

10. When step 9 is almost finished, prepare the following (Fragmentation mix):

    10.0 µl RNase-free H_2O
    4.8 µl of Fragmentation buffer
    1.0 µl of 10 U/µl UDG enzyme
    1.0 µl of 1000 U/µl APE1 enzyme
    Total volume 16.8 µl

11. Vortex briefly and keep on ice until needed.
12. Add 16.8 µl of Fragmentation mix to the cooled sample DNA. Total volume should be 45 µl.
13. Incubate mixture at 37 °C for 1 hour (program AFFY2)

Labeling - Using the AFFY GeneChip WT Terminal Labelling Kit

14. When step 13 is almost finished, prepare the following (Labeling Mix):

    10.0 µl 5x TdT buffer
    2.0 µl TdT
    1.0 µl DNA labelling reagent
    Total volume 15.0 µl

15. Vortex briefly and keep on ice until needed.
16. Add the total volume of labelling mix to the cooled sample mixture from step 13.
17. Incubate at 37 °C for 1 hour (program AFFY3)

Microarray hybridization, post-treatment, scanning, feature extraction

After preparing the biotinylated sample probe, a hybridization mixture is prepared as follows:

60.0 µl sample mixture
11.0 µl warmed (65 °C) 20x Eukaryote Hybridization Control
3.7 µl B2 Oligo control
15.4 µl DMSO
110.0 µl 2X Hybridization Buffer Mix
20.0 µl dH20

The entire labeled sample reaction is denatured at 99 °C, then cooled to 45 °C for 5 minutes. 200 µl is applied to the microarray, then the array is incubated in a hybridization oven in a rotating holder for 16–18 hours at 45 °C. After hybridization, the array is transferred to the fluidics station which performs a post-hybridization wash followed by an automated labeling with streptavidin-phycoerythrin, a fluorescent chromaphore complex that binds to the biotin in the sample probe.

Post-hybridization arrays were scanned in the 3000-7G Affymetrix array imager. This unit scans the barcode of the array, then applies the appropriate colour and resolution settings automatically, scans the array, and downloads the recorded data to the workstation for analysis. Feature extraction takes place automatically following the array scan download.

List of symbols/abbreviations/acronyms/initialisms

APE	Apurinic endonuclease; cleaves DNA adjacent to apurininc sites
APRT	adenine phosphoribosyltransferase
ATCC	American Type Culture Collection; an organization supplying standard microbial strains and samples
BLAST	Basic Local Alignment Search Tool
bp	base pair
DNA	deoxyribonucleic acid
DRDC	Defence Research & Development Canada
HPT	hypoxanthine guanine phosphoribosyltransferase
mM	millimolar
NCBI	National Center for Biotechnology Information (also referred to as Genbank)
PERL	a high level programming language for scanning text files, extracting data, and generating reports from the data
R&D	Research & Development
SNP	single nucleotide polymorphism; a sequence variant at one base position which may be different between populations or individuals
TDT	terminal deoxynucleotidyl transferase

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There is an ongoing requirement for development of high-density or multiplex assays for detection or identification of microbes. There is also a need to develop assays or toolsets that can detect or identify microbial threats without prior knowledge of the target microbe(s) in a given sample. Indeed, some samples that contain no culturable material (e.g. viable but non-culturable cells) will nonetheless contain detectable DNA fragments which might be of value with respect to forensics or attribution of source. For many pathogenic microbes, various specific tests already exist, but there are few general methods wherein a single adaptable tool can be applied to multiple species or to previously uncharacterized organisms. The high-density DNA microarray has the potential to address many of these requirements and thus complements existing identification tools. The microarray platform has for example, the ability to detect microbial DNA that is not a perfect match to known genomic DNA sequences, thus making it possible to detect microbial variants that might otherwise be missed. In this report, the design and preliminary testing of a high-density DNA microarray for the purpose of microbial identification and detection is described.

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microbial genotyping; genomic fingerprinting; detection; identification; microarray

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